Non-osteoporotic post-menopausal women do not have elevated concentrations of autoantibodies against osteoprotegerin

Isabelle Piec[1], Sophie Paddock[1], Christopher J. Washbourne[1], Jonathan C.Y. Tang[1], Julie Greeves[2], Sarah Jackson[2], Stuart Ralston[3], Philip Riches[3], Helen McDonald[4] and William D. Fraser[1]

1- BioAnalytical Facility, Biomedical Research Centre, Norwich Medical School, Faculty of Medicine and Health Sciences, University of East Anglia, Norwich Research Park, Norwich, Norfolk, United Kingdom NR4 7TJ. 2- HQ Army Recruiting and Training Division. Upavon, UK. 3- Rheumatoid Disease Unit, Institute of Genetics and Molecular Medicine. University of Edinburgh. Scotland. 4- , University of Aberdeen, United Kingdom

Corresponding author: i.piec@uea.ac.uk

University of East Anglia
Norwich Medical School

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Introduction: autoantibodies for OPG in the RANK-RANKL-OPG signalling pathway

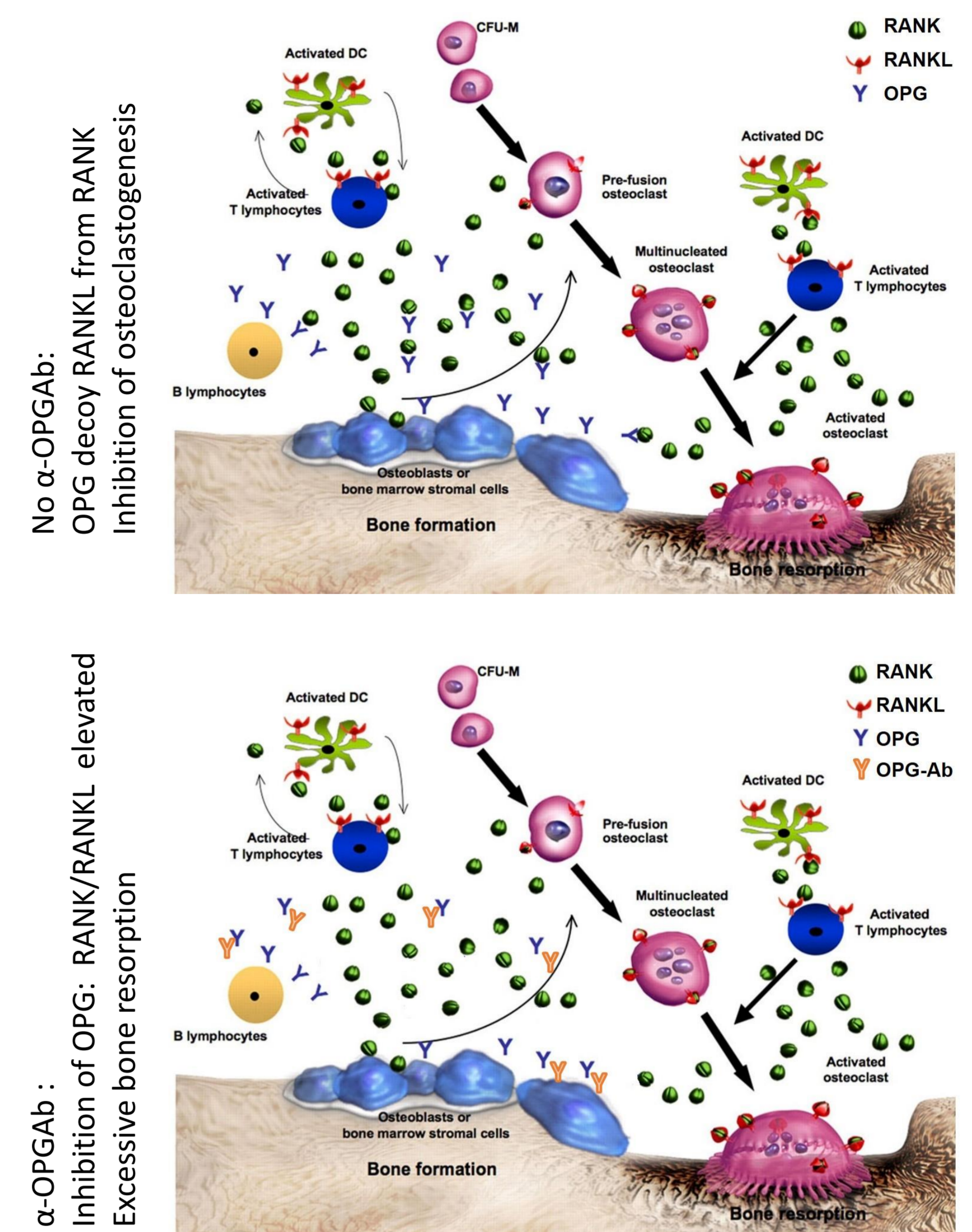The RANK/RANKL/OPG signalling pathway is essential for osteoclastogenesis.

Osteoprotegerin (OPG) is a decoy receptor for RANKL. By binding to RANKL, OPG blocks RANKL-RANK interaction, inhibiting the differentiation of the osteoclast precursor into a mature osteoclast and thereby protecting the skeleton from excessive bone resorption.

- Auto-antibodies against Osteoprotegerin (α-OPGAb), by capturing OPG, enable sustained interaction of RANKL with RANK and over-activation of osteoclasts.
- Such antibodies were identified in 2009, in a man with coeliac disease associated with severe osteoporosis[1] and later in 2013, in patients presenting with rheumatoid arthritis, systemic lupus erythematosus, spondyloarthritis and osteoporosis[2].
- These findings suggest a role for α-OPGAb as primary cause of high bone turnover.

We developed an enzyme linked immunosorbent assay (ELISA) for detection and quantification of α-OPGAb in patient serum samples[3] showing α-OPGAb to be present in 14% of an apparently healthy young adult population.

Bone resorption is increased in the elderly, particularly in women who may demonstrate increased α-OPGAb.

We aimed to define a reference range for OPG autoantibodies in non-osteoporotic post-menopausal women.

RANK/RANKL/OPG signalling pathway and role of α-OPGAb.
Adapted from Boyle WJ, *et al. Nature* 2003 423(6937):337-42[5]

Method: α-OPGAb assay on serum samples

❖ **Samples**
- Serum samples from non-osteoporotic 60-65yr-old post-menopausal women (ANSAVID study[4], n=134)
- Serum samples from healthy volunteers following and in accordance with the Ministry of Defence Research Ethics Committee (MODREC-165) (18-26yrs, n=51).

❖ **ELISA:**
- Plates (Maxisorp*, ThermoFisher Scientific)* were coated with 0.5µg/mL recombinant OPG (*Novoprotein*)
- Samples/standards (rabbit OPGab, *Abnova*) and controls (50µL) were incubated for 3hrs at RT
- A two-step detection was used: goat polyclonal biotin conjugated anti-human OPG antibody (*ThermoFisher Scientific*) and Streptavidin conjugated horseradish peroxidase (*Jackson ImmunoResearch*).
- TMB (Sigma Aldrich) was used as substrate and signal was measure using a Multiskan software linked to a plate reader (*ThermoFisher Scientific*). Circulating antibody concentration is calculated against a 4-Parameter Logistic equation (Typical obtained using a polyclonal r^2=0.9916).
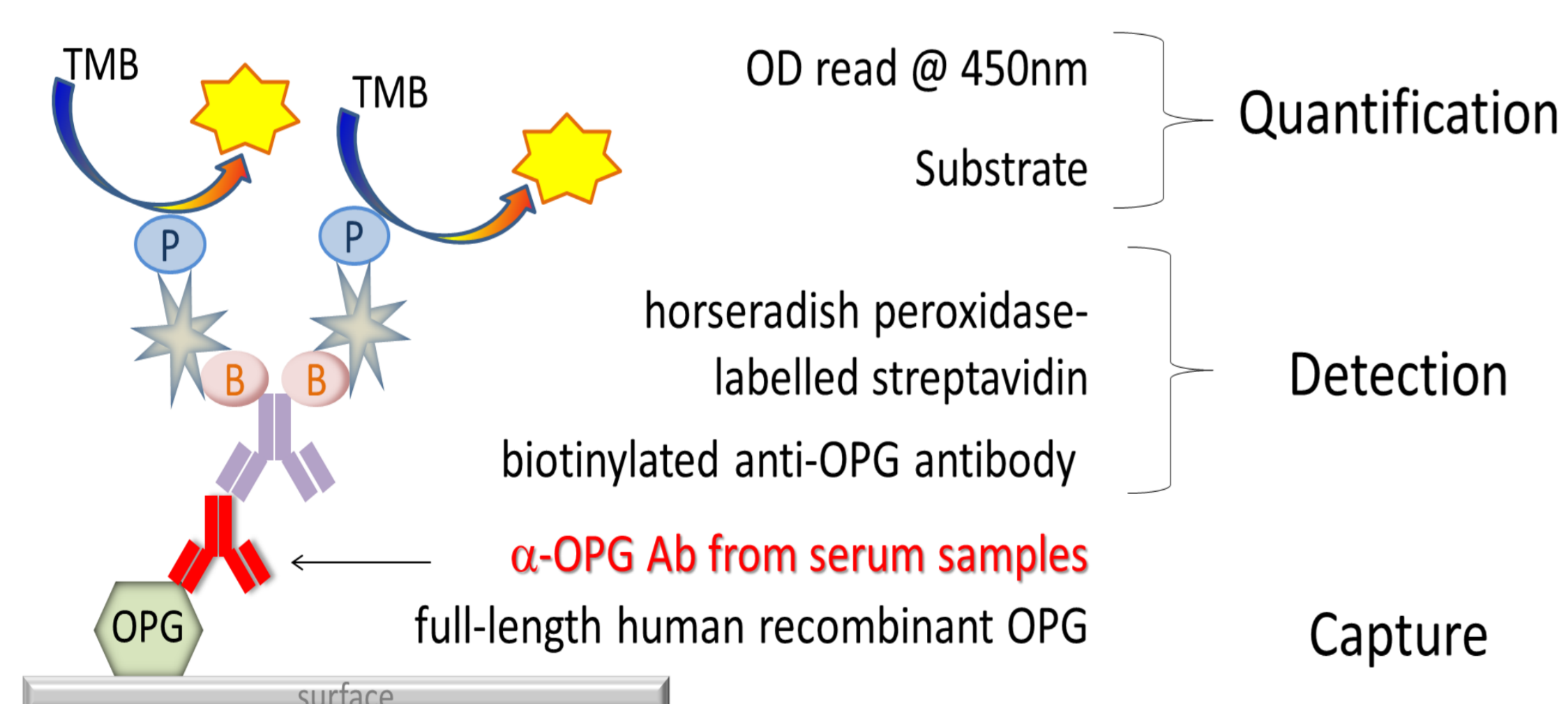
Schematic of α-OPGAb assay principle.

Results: Distribution of α-OPGAb

❖ Skewed distribution of α-OPGAb in both populations
❖ Adult population would be considered positive with a titer above the cut-off limit (95%) of 191ng/mL calculated using the geometric mean of log10 dataset
❖ The reference ranges obtained were 134-191ng/mL and 131-184ng/mL for control and post-menopausal women, respectively.
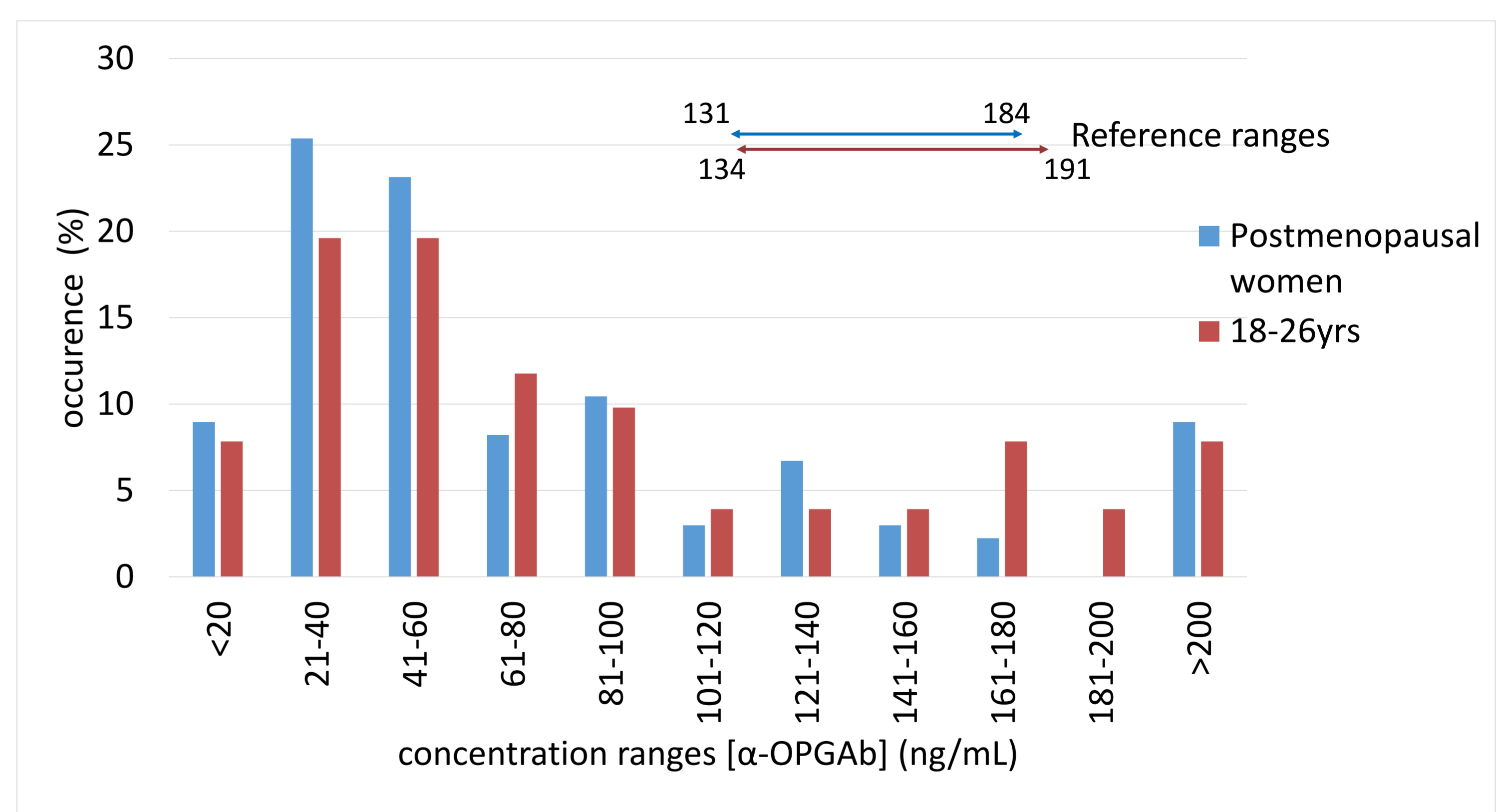
Distribution of samples α-OPGAb concentration in healthy young (red) and postmenopausal (blue) women

Conclusions

We established that the population of normal post-menopausal women who do not have osteoporosis also do not have elevated concentrations of α-OPGAb when compared to a younger female population (18-26 yrs). This suggest that α-OPGAb is not positively associated with increasing age suggesting that the increased production of α-OPGAb is mainly related to pathologic conditions which can result in significant bone resorption.
Comparison of osteoporotic patient samples to non-osteoporotic post-menopausal women would be interesting to determine whether α-OPGAb can be used to detect patients at high risk of bone resorption and identify appropriate treatment for this particular subgroup of patients.
We are designing a humanized antibody against human OPG in order to eliminate false positive.

References:
1-Riches et al. (2009) N Engl J Med:361 pp1459-65. **2-**Hauser et al. (2013) Bone Abstracts: 1 pp383. **3-**Piec *et al.* (2014) Poster presented at: American Society for Bone and Mineral Research conference. **4-**Macdonald *et al.* (2011) JECM: 96 pp1677-86. **5-** Boyle et al. (2003) Nature:423 pp337-42